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GENERAL
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A Case of Anomalous Chloroplast Inheritance in Crosses of Garden Pea Involving an Accession of Wild Subspecies

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In a series of crosses of various *Pisum sativum* L. forms, we met a sample collected by N.I. Vavilov in Palestine, VIR320, a wild subspecies *P. sativum* ssp. *syriacum* (Boiss et Noe) Berger, with unusual behavior [1]. When VIR320 was used in crosses as a female parent, essentially all F1 plants were aberrant. Chlorophyll pigmentation of these plants was disturbed; leaflets and stipules were markedly reduced; fertility was strongly suppressed, so that most of plants did not produce seeds; plants usually branched intensely. When VIR320 plants were used in crosses as a male parent, the progeny was normal. Such asymmetry in the plant phenotype in reciprocal crosses indicates clearly the occurrence of nuclear–cytoplasmic incompatibility resulting in the damage to chloroplast functions. This conflict can be overcome: some plants have leaf sectors with normal pigmentation; sometimes, entire branches are indistinguishable from normal ones. In this study, we demonstrated that the recovery of normal chloroplast functioning in the presence of nuclear–cytoplasmic conflict is possible by proliferation of paternal plastids, which is an obvious deviation from normal maternal inheritance of cell organelles.

The phenotype of plants in our experiments resembled markedly the phenotype described by A.N. Lutkov [2], which he observed in F2 and F3 of *P. sativum* × *P. humile* Boiss et Noe hybrids. This permitted L.I. Govorov [3] to consider *P. humile* as a separate species. Later, the name *humile* was replaced by *syriacum,* and the taxon itself was considered as a subspecies of garden pea *P.* s. ssp. *syriacum.* [4]. At present time, a simple taxonomic schema was elaborated, according to which all wild forms of garden pea belong to a single subspecies *P. sativum* ssp. *elatius* (Bieb.) Schmahl. This classification was used in the study [1] as well.

In this work, we analyzed the marker of chloroplast DNA in F1 plants resulting from reciprocal crosses of VIR320 line with pea tester lines Sprint-1 and RT-1 produced in our laboratory. Sprint-1 line was obtained by crossing cv. Avanti and an accession VIR7036 (Nepal); in RT-1 line production, Sprint-1 line, WL1018 test-line, and VIR320 accession were involved. The latter line was studied in this work as a carrier of genetically determined capability of induction of disturbances in hybrid progeny.

The *rbcL* gene, encoding Rubisco large subunit, was used as a marker of chloroplast DNA. A 1129-bp fragment of the coding region of this gene was amplified using the forward primer 5'-TTATTATACTCCTGACTATCAAACC-3' and the reverse primer 5'-TACAGAATCATCTCCAAATATCTCG-3'. These primers were constructed based on the published nucleotide sequence of pea *rbcL* (accession no. X03853)[1] PCR conditions were as follows: preliminary denaturing at 95°C for 1 min, then 35 cycles including denaturing at 94°C for 59 s, annealing at 62°C for 59 s, and extension at 72°C for 1 min. Total DNA was extracted from F1 plants using a kit for DNA isolation (Medigen, Novosibirsk). For PCR amplification of the *rbcL* gene, about 10 ng of DNA was used. The product obtained was treated with AspLEI restrictase (Sibenzyme, Novosibirsk) and electrophoresed in 1.5% agarose gel containing 0.5 μg/ml of ethidium bromide.

It turned out that the amplified fragment from VIR320 line had a site recognized by the AspLEI endonuclease, so that two fragments were produced after restriction, about 800 and 300 bp in length. This site was absent from Sprint-1 and RT-1 line *rbcL* genes; their PCR products were not digested and had a length of about 1100 bp. Sequencing of the gene region showed that the appearance of this restriction site occurred due to the replacement of T with C. We analyzed 10 F1 plants derived by VIR320 × Sprint-1 cross. All plants manifested signs of nuclear–cytoplasmic

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[1] DNA sequence was determined at the Center for DNA sequencing organized on the base of the Institute of Cytology and Genetics and the Institute of Chemical Biology and Fundamental Medicine, Siberian Division, Russian Academy of Sciences.

incompatibility: disturbances in chlorophyll pigmentation and reduction of leaflet blades and stipules (Fig. 1). Three of these plants overcame partially this conflict and had sectors of normal tissue (Fig. 2). In garden pea, maternal inheritance of chloroplasts was shown [5]. Therefore, we expected to find two DNA fragments (about 800 and 300 bp) because of the presence of the restriction site in VIR320 DNA. However, almost in all plants, we noted some degree of heteroplasmy. Seven plants did not manifest any signs of overcoming of the nuclear–cytoplasmic conflict and had a maternal type of *rbcL*-encoding chloroplast DNA with some traces of the paternal type (Fig. 3, lanes 2, 5–9, 11). In three plants with sectors of normal tissue, the predominance of paternal type of chloroplast DNA was noted (Fig. 3, lanes 3, 4, 10). (Here and later, speaking about maternal or paternal type of chloroplast DNA, we mean the *rbcL* marker gene.) In one of the plants with sectors of normal tissue (Fig. 2), we analyzed DNA isolated from green regions and from chlorotic parts. It turned out that chlorotic parts had mainly the maternal type, whereas green parts, the paternal type of chloroplast DNA (Fig. 4). Based on these data, we concluded that plastids derived from VIR320 line could not function normally in the cells where the half of the nuclear genome was represented by the Sprint-1 DNA. This is manifested in the disturbance of chlorophyll pigmentation and accompanying reduction of the leaf structures. The restoration of the green tissue occurs when paternal chloroplasts proliferate, which is an atypical phenomenon for peas but is in agreement with a potential capability of biparental chloroplast inheritance in this species [6, 7].

When VIR320 line was crossed with RT-1 line, all progenies had normal green coloration. That means that, in this case, there was no conflict between the nucleus and cytoplasm, which might be explained by genetic similarity of these lines. The analysis of the *rbcL* gene in five F1 progenies showed that the predominant amount of DNA was represented by the maternal type; however, the traces of the paternal variant were noted in one plant. Maternal inheritance of chloroplast DNA was observed also in reciprocal crosses RT-1 × VIR320 and Sprint-1 × VIR320 (Fig. 3, lanes 13–16), when VIR320 line was a pollen donor. Thus, in the absence of the nuclear–cytoplasmic conflict, we observed an expected [5] maternal chloroplast inheritance.

Similar anomalies in the inheritance of cytoplasmic genomes were described for the intergeneric crosses of cereals. In F1 hybrids between rye and triticale, the analysis of mitochondrial DNA detected the presence of both maternal and paternal fragments, whereas these hybrids manifested strictly maternal inheritance of chloroplasts [8]. In hybrids of barley and wheat, heteroplasmy of the markers of mitochondrial DNA was detected in the series of backcrosses; fertility restoration, as an indicator of overcoming the nuclear–cytoplasmic conflict, was correlated with the inheritance of only paternal variant [9]. While paternal variant of mitochondrial DNA was found already in F1 hybrids, paternal variant of chloroplast DNA appeared only in the third or fourth generation of backcrosses [10], i.e.,

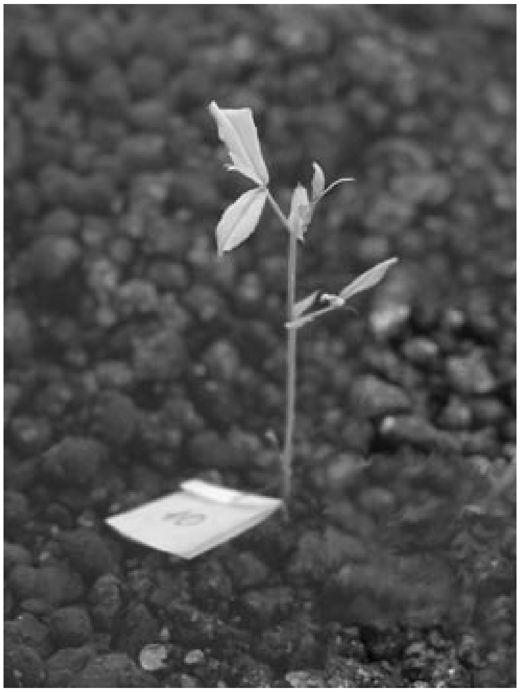

Fig. 1. Phenotype of a typical F1 plant of the cross VIR320 × Sprint-1.

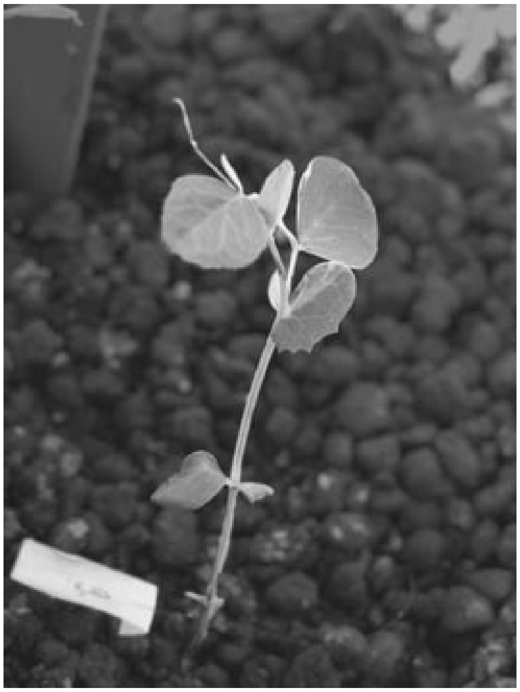

Fig. 2. Phenotype of an F1 plant of the cross VIR320 × Sprint-1 having overcome the nuclear–cytoplasmic conflict.

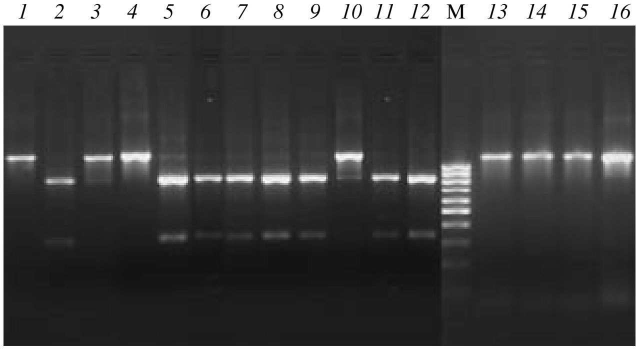

Fig. 3. Fragment of the *rbcL* gene coding region after digestion with AspLEI restrictase. (*1*) A parent form Sprint-1; (*2–11*) individual F1 plants of the cross VIR320 × Sprint-1; (*12*) a parent form VIR320; (M) markers of mol wts 100–1000 bp; (*13–16*) individual F1 plants of the cross Sprint-1 × VIR320.

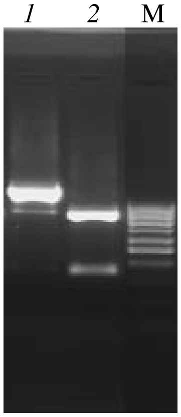

Fig. 4. Fragment of the *rbcL* gene coding region after digestion with AspLEI restrictase. DNA was isolated from a single F1 plant. (*1*) DNA from green leaf parts; (2) DNA from chlorotic leaf parts; (M) markers of mol wts 100–1000 bp. In Fig. 3 this plant is presented in lane 10.

at more complete saturation of the genome of distant hybrids with the paternal genetic material.

We observed paternal inheritance of chloroplast DNA in pea hybrids, which was correlated with the restoration of normal green pigmentation of the mesophyll and of total plant vitality. This phenomenon differed from those described for distant cereal hybrids in that, firstly, it occurred during intraspecies hybridization (subspecies crosses) and, secondly, anomalies in chloroplast inheritance were observed already in the first generation.

It is worth mentioning that the VIR320 accession was highly heterogeneous [4]. The seed material we received from VIR contained two rather dissimilar forms, and the anomaly described was characteristic of only one of them.

It is pertinent to note that crosses of *Pisum sativum* with subspecies *P. s.* ssp. *abyssinicum* and species *Pisum fulvum* were successful (with rare exception) only when *Pisum sativum* was used as a female parent [11, 12]. It is possible that the nuclear–cytoplasmic conflict contributes to the formation of reproductive barrier preventing crosses and to the species differentiation in the genus *Pisum.*

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